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Optical Control of Metabotropic Glutamate Receptors for Probing of G Protein Signaling and Receptor Activation Mechanism

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G-protein coupled receptors (GPCRs) are the largest family of membrane signaling proteins. They respond to a wide-array of stimuli and contain a seven transmembrane domain that couples to heterotrimeric G-proteins. These pathways play roles in controlling second messenger levels and regulating other signaling proteins, such as ion channels and kinases. Due to their importance in many diseases, GPCRs are the most explored drug targets in biology. Despite this, the specific biochemical, physiological, and behavioral roles of many GPCRs are not well-understood and make this field ripe for the application of new tools for high-precision probing. We have developed an approach to elucidate the function of a GPCR by chemically re-engineering it to be sensitive to light. The class C glutamate-gated GPCR, mGluR2, which couples to the Gi/o pathway, was derivatized with photoswitchable ligands to generate both light-agonized (LimGluR2) and light-antagonized (''LimGluR2-block'') receptors. The bistable, azobenzene photoswitch enables activation by a light pulse to be sustained for long periods in the dark before being switched off by a longer wavelength light pulse. LimGluR2 deactivates quickly and supports multiple reproducible rounds of on/off switching with superior fidelity and speed compared to Rhodopsin. We have extended optical control to a variety of mGluRs with distinct G-protein coupling profiles. These designed receptors provide excellent tools for the dissection of the specific roles of different mGluRs in physiological functions, such as induction of synaptic plasticity, with high spatiotemporal precision. Furthermore, the high level of control afforded by tethered agonists allows for the probing of coupling of ligand-binding to receptor activation with single-subunit control. Along with single-molecule fluorescence experiments, we have used optical control via LimGluRs to probe the mechanisms of assembly and cooperative activation in the class C GPCRs.

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Deciphering the Cross-Talk of the Prostaglandin G-Protein Coupled Receptors EP2 and EP4: From Molecular Insights to Novel Anti-Tumor Targets

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Prostaglandin E2 (PGE2) favors cancer progression by promoting angiogenesis and metastasis, and by influencing the immune response, thus being a key tumorigenic factor. The specificity and diversity of PGE2 effects can be explained by cells expressing a characteristic pattern of different EP receptors with distinctive signaling mechanisms. PGE2 signaling through both EP2 and EP4 receptors coupled to the G-protein Gαs is known to stimulate cAMP production. Interestingly, although details of the down-stream signaling pathways remain unknown, increasing evidence suggests that EP4 also couples to Gαi, which is known to inhibit cAMP production, thus triggering a natural inhibitory pathway. We aim to unravel the dynamics and molecular mechanisms of the cross-talk of EP2 and EP4 receptors via the inhibitory Gαi and the stimulatory Gαs signaling axis at the molecular level. FRAP and FLIM studies confirmed that only EP4 signals via Gαi and showed that although the intitiation of PGE2-induced Gαs and Gαi signaling occurs simultaneously, Gαs signals longer than Gαi. Single-particle tracking of q-dot labeled receptors revealed that EP4 slows down upon PGE2 stimulation whereas EP2 only decreases mobility when EP4 is present, suggesting cross talk. Furthermore, using a cAMP FRET-sensor we were able to investigate the extent of cAMP production using EP2 and EP4 specific agonist/antagonist and showed that EP4 signaling caused a transient cAMP response. We propose a model where EP4 allows for the fine-tuning of the cell's response to PGE2, providing a threshold for a cell to respond to PGE2, whereas EP2 continuously signals to high levels of PGE2. We believe that targeting PGE2 signaling and control the outcome of the cell's response to PGE2 will provide new tools for the development of alternative therapeutic approaches to cancer treatment.

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Dopamine D2 Receptor Antagonist Unbinding Rates Investigated using a Time-Resolved Ion Channel Activation Assay

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The lower liability of atypical antipsychotics to produce side-effects correlates with their faster rates of dissociation from the dopamine D_2 receptor. Recent studies indicate that the novel D_2 ligands, ACR16 and OSU6162, act as antagonists with similarly high dissociation rates. However, those studies measured dissociation of radiolabeled ligand from membrane preparations or used modified G proteins to study calcium release. We examined relative antagonist dissociation rates in living cells, using a time-resolved assay based on activation of G protein-coupled potassium channels (GIRK) by native G proteins. GIRK responses to dopamine receptor activation were studied using two-electrode voltage clamp in Xenopus oocytes expressing D_2 receptors and GIRK. First, dopamine was applied, resulting in a ''baseline'' response. Next, antagonist was washed in, in the continued presence of dopamine. After attaining steady-state response inhibition, antagonist was washed out, still in the presence of dopamine. Response recovery was recorded over six minutes, and the time-course and relative amplitude of recovery were taken as measures of antagonist dissociation. Significant differences in response recovery T1/2 and recovery amplitudes were observed between the different D_2 receptor antagonists: In experiments with haloperidol, risperidone, and aripiprazole, virtually no response recovery was observed. With clozapine and quetiapine, similar recovery amplitudes and recovery time courses were observed (T1/2 ~ 40 s). ACR16 and OSU6162 behaved as antagonists, lacking detectable efficacy in the GIRK assay. These compounds washed out with similar time courses (T1/2 ~ 8 s); significantly faster than the other antagonists. ACR16 and OSU6162 appear to dissociate faster than clozapine and quetiapine, a finding which has not been reported earlier. Such very rapid dissociation might be relevant to the low incidence of side effects reported from clinical trials with these compounds.

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Voltage-Dependent Modulation of α_{2A} Adrenergic Receptor Conformations

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G protein-coupled receptors (GPCRs) are proteins that span the cell membrane seven times. They are stimulated by extracellular agonists and activate heterotrimeric G proteins to elicit intracellular responses. Recent evidence suggests that GPCRs are voltage-sensitive: They exhibit gating currents similar to ion channels and respond to depolarization of the plasma membrane with changes in agonist affinity. We used a FRET-based biosensor of the α2A adrenergic receptor to analyze voltage dependence of receptor activation in HEK 293 cells by means of voltage-clamp recording. The biosensor was stimulated either with the partial agonist clonidine or with the full agonist norepinephrine (NE) and receptor activation was measured as decrease in FRET ratio. Receptor stimulation by NE was reduced by membrane depolarization and enhanced by hyperpolarization. This effect was present in wilde-type receptors and transduced to the level of G protein activation, which we determined in a FRET assay that directly detects Gαi protein activation. Depolarization-mediated inhibition of NE activated receptors was strong at low concentrations (500 nM: 60 % inhibition) but almost undetectable at saturating agonist concentrations (100 μM: 9 % inhibition). Both agonist-induced and hyperpolarization-induced receptor activation exhibited a similar monoexponential time course. For both activation modes, speed of activation was primarily dependent on agonist concentration, indicating that depolarization lowers the apparent affinity of the NE receptor interaction and thus causes receptor deactivation by means of NE release. Application of clonidine (1 μM, VM=-90 mV) resulted in a FRET response that was inhibited by 40 % at +60 mV. In contrast to NE, strong receptor-inhibition at +60 mV was present even at super-saturating concentrations of clonidine (100 μM). Therefore we conclude that negative membrane potentials promote active conformations of the α2A adrenergic receptor, increase affinity of full agonists and enhance G protein signaling.